

For the convenience of the Examiner, a marked-up copy of the amended paragraphs from the specification is attached as an Appendix. Nevertheless, the material as set forth above governs in case of any inconsistency.

New claims 25-42 are pending in the present application. Claims 25-42 are directed to purified collagenase inhibitor proteins, recombinant vector systems comprising DNA encoding a collagenase inhibitor protein, and recombinant DNA methods for making collagenase inhibitor proteins, as well as recombinant DNA methods for microbial production of collagenase inhibitors with vector pUC9-F5/237P10. These claims correspond directly to, or are derived from, earlier-filed claims in this series.

Specifically, claim 25 is drawn to the protein encoded by DNA sequences recited by allowed claim 25 in the '817 application. Claim 26 corresponds to claim 84 as filed in U.S. Application No. 08/474,553 on November 25, 1996. Claims 27 and 28, which recite plasmid or viral vectors that encode the collagenase inhibitor proteins of claims 25 and 26, and claim 42, which is drawn to a host microorganism comprising these vectors, are supported, for example by the claims as originally filed in U.S. Application No. 06/784,319 ("the '319 application"). Claim 33 and claims 35-41 which depend therefrom, which recite recombinant DNA methods for the microbial production of a collagenase inhibitor, wherein a host organism contains the vector pUC9-F5/237P10, find support, for example in claims 11-19 of the '319 application.

Although a Sequence Listing is not required in the instant application, Applicants have voluntarily complied with the Sequence Listing requirements by enclosing a paper copy of the Sequence Listing filed in parent U.S. Application No. 09/452,817, on

June 22, 2001. Applicants hereby request that the previously submitted computer readable copy of the Sequence Listing be associated with and used in the instant case.

The undersigned hereby states that the attached paper copy is identical to the computer readable copy of the Sequence Listing submitted on June 22, 2001, in the parent application, U.S. Patent Application Serial No. 09/452,817.

Applicants respectfully request the examination of this application and the timely notification of the allowability of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to our Deposit Account No. 06-0916.

Respectfully submitted,

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**APPENDIX: Marked-up version**

**In the Specification:**

The paragraph on page 4, beginning at line 4, has been amended as follows:

--Surprisingly, the present inventors have discovered a portable DNA sequence capable of directing the recombinant-DNA synthesis of metalloproteinase inhibitors. These metalloproteinase inhibitors are biologically equivalent to those isolated from human skin fibroblast cultures. The metalloproteinase inhibitors of the present invention, prepared by the recombinant DNA methods set forth herein, will enable increased research into prevention and treatment of metalloproteinase-induced connective tissue diseases. In addition, the metalloproteinase inhibitors of the present invention are useful in neutralizing [metalloproteinases] metalloproteinases, including the excess metalloproteinase associated with disease states. Therefore, it is believed that a cure for these diseases will be developed which will embody, as an active ingredient, the metalloproteinase inhibitors of the present invention. Furthermore, the metalloproteinase inhibitors of the present invention are capable of interacting with their metalloproteinase targets in a manner which allows the development of diagnostic tests for degradative connective tissue diseases using the newly discovered inhibitors.--

The paragraph on page 7, beginning at line 11, has been amended as follows:

--The coding strand of a first preferred DNA sequence which has been discovered has the following nucleotide sequence (SEQ ID No: 5):--

The paragraph on page 8, beginning at line 12, has been amended as follows:

--A second preferred DNA sequence has been discovered which has an additional nucleotide sequence 5' to the initiator sequence. This sequence, which contains as the eighty-second through four-hundred-thirty-second nucleotides nucleotides 1 through 351 of the first preferred sequence set forth above, has the following nucleotide sequence (SEQ ID No: 6):--

The paragraph on page 9, beginning at line 5, has been amended as follows:

--A third preferred DNA sequence which incorporates the 5' region of the second preferred sequence and the 3' sequence of the first preferred sequence, has the following nucleotide sequence (SEQ ID No: 7):--

The final paragraph on page 13 has been amended as follows:

--A first preferred portable DNA sequence of the present invention has a nucleotide sequence SEQ ID No: 5 as follows:--

The paragraph on page 15, beginning at line 6, has been amended as follows:

--A second preferred portable DNA sequence of the present invention has the following nucleotide sequence (SEQ ID No: 6):--

The final paragraph on page 15 has been amended as follows:

--In this second preferred sequence, an open reading frame exists from nucleotides 1 through 432. The first methionine of this reading frame is encoded by nucleotides [by] 49 through 51 and is the site of translation initiation. It should be noted that the amino acid sequence prescribed by nucleotides 49 through 114 is not found in the mature metalloproteinase. It is believed that this sequence is the leader peptide of the human protein.

The sentence on page 16, beginning at line 3, has been amended as follows:

--A third preferred portable DNA sequence has the [mucleotide] nucleotide sequence (SEQ ID No: 7):--

The nucleotide sequence on page 28, beginning on line 14, has been amended as follows:

HgiAI  
--(SEQ ID No: 8) [5'] 5' GAT CCG TGC ACT TGT GTT CCA CCA CAC  
(SEQ ID No: 9) GC ACG TGA ACA CAA GGT GGT GTG  
CCA CAA ACT GCT TTC TGT AAC TCT GAC C  
GGT GTT TGA CGA AAG ACA TTG AGA CTG GA 3'--

The paragraph on page 35, beginning at line 13, has been amended as follows:

--In this method, the portable DNA sequences are those synthetic or naturally-occurring polynucleotides described above. In a preferred embodiment of the present method, the portable DNA sequence has the nucleotide sequence SEQ ID No: 5 as follows:--

The paragraph on page 39, beginning at line 18, has been amended as follows:

--In certain circumstances, the metalloproteinase inhibitor will assume its proper, active structure upon expression in the host microorganism and transport of the protein through the cell wall or membrane into the periplasmic space. This will generally occur if DNA coding for an appropriate leader sequence has been linked to the DNA coding for the recombinant protein. The preferred metalloproteinase [metalloproteinase] inhibitors of the present invention will assume their mature, active form upon translocation out of the inner cell membrane. The structures of numerous signal peptides have been published, for example by Marion E.E. Watson in Nuc. Acid Res. [12: 515-5164] 12: 5145-5164, 1984, specifically incorporated herein by reference. It is intended that these leader sequences, together with portable DNA, will direct intracellular production of a fusion protein which will be transported through the cell membrane and will have the leader sequence portion cleaved upon release from the cell.--

The paragraph on page 41, line 10, has been amended as follows:

--Preparation of Poly(A<sup>+</sup>) [RNS] RNA from HEF-SA Fibroblasts--

The paragraph on page 56, beginning on line 10, has been amended as follows:

--The structure of FIBAC A is

(SEQ ID No: 10) GA TCC GCG ATC GGA GTG TAA GAA ATG TGC ACT  
(SEQ ID No: 11) G CGC TAG CCT CAC ATT CTT TAC ACG TGA  
  
TGC GTT CCG CCG CAT CCG CAG ACT GCT TTC  
ACG CAA GGC GGC GTA GGC GTC TGA CGA AAG  
  
TGC AAC TCT GAC C  
ACG TTG AGA CTG GA--

The paragraph on page 56, beginning on line 19, has been amended as follows:

--Component oligonucleotide FA1 (SEQ ID No: 12) is:  
GATCC GCGAT CGGAG TGTAA GAAAT GTGCA CTTGC--

The paragraph on page 56, beginning on line 21, has been amended as follows:

--Component oligonucleotide FA2 (SEQ ID No: 13) is:  
GGAACG CAAGT GCACA TTTCT TACAC TCCGA TCGCG--

The paragraph on page 56, beginning on line 23 has been amended as follows:

--Component oligonucleotide FA3 (SEQ ID No: 14) is:  
GTTC CGCCG CATCC GCAGA CTGCT TTCTG CAACT CTGAC C--

The paragraph on page 56, beginning on line 25, has been amended as follows:

--Component oligonucleotide FA4 (SEQ ID No: 15) is:  
AGGTC AGAGT TGCAG AAAGC AGTCT GCGGA TGCAG C--

The sentence on page 57, line 4, has been amended as follows:

--Linker A1 (SEQ ID No: 16) is: AATTGGCAG--

The sentence on page 57, line 5, has been amended as follows:

--Linker A2 (SEQ ID No: 17) is: TCGACTGCC--

The first sentence on page 58 has been amended as follows:

--The sequence of the sense strand (SEQ ID No: 18) is:--

The sentence on page 59, line 11, has been amended as follows:

--Linker B1 (SEQ ID No: 19) is: GATCCCAGGCCTGCA--

The sentence on page 59, line 12, has been amended as follows:

--Linker B2 (SEQ ID No: 20) is: GGCCTGG--

The sentence on page 68, line 4, has been amended as follows:

--The second preferred sequence (SEQ ID No: 6) as set forth herein, i.e.,--

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